Communication

Photoactivatable Substrates for Analyzing Cell Migration

Jun Nakanishi

World Premier International (WPI) Research Center Initiative, International Center for Materials Nanoarchitectonics (MANA), National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan
NAKANISHI.Jun@nims.go.jp

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1. Introduction

Migration is a fundamental biological activity of cells, which is important for various physiological processes such as morphogenesis, wound healing and immune responses [1]. On the other hand, cell migration is also involved in pathological processes, like cancer invasion and metastasis. Therefore, understanding migratory behavior of single cells and cell clusters is important not only for fundamental biological interests, but also for therapeutic applications.

Various methodologies have been developed to study cell migration in vitro. For example, scratch wound healing assay [2] is widely used in laboratory to explore the role of particular genes or posttranslational modification in cell migration. Boyden chamber assay is suitable for the evaluation of the activities of soluble molecules in promoting or retarding cell migration in a high throughput manner. A wide range of internal and external factors has been identified to regulate cell motility by these techniques. However, most of these studies are focusing on extracellular soluble factors and intracellular signaling molecules, and little attention has been paid on the contribution of cellular micro- or nanoenvironments composed of extracellular matrices (ECM) and surrounding cells.

To address this issue, we developed photoactivatable substrates, which changed from non-cell-adhesive to cell-adhesive in response to light (Figure 1a), and applied them for studying cell migration (Figure 1(b)) [3]. One of the major advantages of this method is that cellular microenvironments can be photochemically controlled by using a standard fluorescence microscope. Here, the author explain an example of the design rationale and working principle of a photoactivatable substrate and introduce an application of such substrate for analyzing cell migration.

2. Design rationale and working principle

Common design rationale of our photoactivatable substrates is to physically or chemically conjugate a cell repellent polymer, basically poly(ethylene glycol) (PEG), to the substrate surface via a photocleavable 2-nitrobenzyl group (Figure 1(a)) [4]. The polymer prevents cell adhesion to the surface, but the surface becomes to permit cell adhesion after releasing the polymer by near-UV irradiation. On
these substrates, we are able to not only confine the cells within the irradiated spots (cellular patterning) but also induce their migration by the secondary irradiation (Figure 1(b)). The driving force of cell migration is the same as that of conventional scratching wound healing assay, but the present approach has a big advantage in studying cell migration at the leading edge, as we can precisely control initial cellular pattern and exclude the effect of cell debris created during the scratching process.

Figure 2 shows the chemical structure of a photoactivatable substrate. It is prepared by functionalizing an amino-terminated substrate with PEG bearing a photocleavable succinimidyl ester \([5,6]\). It should be noted that, the original amino-terminated substrate is regenerated without residual chemical modification after photocleavage of PEG. This reversible PEGylation reaction is of great importance because the amino group is basically well cell-adhesive. In addition, it imply for potential applications for studying cell migration on protein-based scaffolds, as the amino group is abundantly existed in biological substances.

3. Application of a photoactivatable substrate for the analysis of the effect of cellular microenvironments on collective migration

In epithelial tissues, cells are interconnected and exhibit sheet-like coordinated migration upon wound healing. It has been reported that the cells at the wound edge are separated into fast moving “leader cells” with active lamellipodia protrusions and the “follower cells” with little or no lamellipodia activity (Figure 3(a)). But it remains poorly understood which cell-internal and external factors regulate this separation. We used a photoactivatable substrate to tackle this issue \([7]\). Epithelial Madin-Darby canine kidney (MDCK) cells were confined within circular regions with different sizes and cell migration was induced by the secondary flood exposure of the substrate (Figure 3(b)). Leader cells appearance per unit perimeter decreased as increasing the cluster size and the initial incubation time within the cluster. The results strongly suggest collective characteristics are highly dependent on cellular microenvironments.

4. Conclusions

Photoactivatable substrates have been developed for the spatiotemporal control of cell migration. These materials are useful for the precise control of cellular microenvironments and provide new methodology for analyzing cell migration.

References